

oligomeric pore capable of cytochrome *c* release. The biophysical mechanism of BAX activation is controversial and several *in vitro* and *in vivo* methods of its activation are known. One of the most commonly used *in vitro* methods is activation with non-ionic detergents, such as *n*-octylglucoside. During BAX activation with *n*-octylglucoside, it has been shown that BAX forms high molecular weight complexes. These complexes are ascribed to the oligomerization of BAX prior to membrane insertion and pore formation. This is in contrast with the *in vivo* studies which suggest that in cells active BAX inserts into the OMM as a monomer and then undergoes oligomerization to form a pore. Here, we used an approach which combines three single-molecule sensitivity techniques - fluorescence correlation spectroscopy (FCS), fluorescence cross-correlation spectroscopy (FCCS) and fluorescence-intensity distribution analysis (FIDA). We used FCS to determine the apparent molecular weight of the BAX-detergent micelles. The FCCS was used to determine the presence of BAX homo-oligomers in detergent micelles, while FIDA was used to determine the oligomerization number of BAX in detergent micelles. We have tested a range of detergents: *n*-octylglucoside, dodecylmaltoside, Triton X-100, Tween 20, CHAPS and cholic acid. With these detergents we consistently observe that BAX is a monomer before, during and after interaction with micelles. We conclude that detergent activated BAX is a monomer and that in physiological buffer conditions BAX can assume two stable monomeric conformations: one inactive and one active. This conclusion is in agreement with the *in vivo* mechanism of BAX induction of apoptosis.

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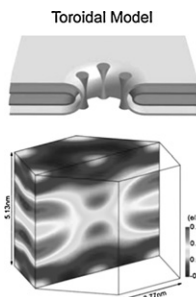
Evidence for Lipidic Pores

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This study revealed the structure and mechanism of pore formation in membranes by Bax- $\alpha 5$ peptide, a segment from the pore-forming domain of Bax. Bax is an apoptosis regulator protein that forms pores in the outer-mitochondrial membrane to release cytochrome-*c*. Bax- $\alpha 5$ has been shown to reproduce the pore-forming activity of Bax. Bax- $\alpha 5$ induced pores in multiple bilayers were long-ranged correlated into a periodically ordered lattice and analyzed by X-ray anomalous diffraction. The electron density profile unambiguously shows the Bax- $\alpha 5$ pore is of the toroidal (wormhole) type: the two lipid monolayers merge through the pore. This was the first direct structural evidence for the existence of the long speculated lipidic pores.

The molecule mechanism of Bax- $\alpha 5$ pore formation was studied by two experiments: pore formation in individual GUVs exposed to Bax- $\alpha 5$ in solutions and the membrane thinning effect caused by the peptides. Bax- $\alpha 5$ exhibited a sigmoidal concentration dependence similar to antimicrobial peptides we've studied: below a threshold concentration, the peptide only binds to membrane inter surface, causing membrane thinning; when the concentration exceeds a critical value, pore formation is activated. Our results suggest that formation of such lipidic pores is a major mechanism for α -pore-forming peptides and proteins.



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Apoptosis Induction is Associated with VDAC Oligomerization

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Mitochondria-mediated apoptosis involves efflux of a number of potential apoptotic regulators, such as cytochrome *c*, to the cytosol, triggering the caspase cascade and cell destruction. The precise mechanism regulating cytochrome *c* release remains unknown, and the molecular architecture of the cytochrome *c*-conducting channel has also to be determined. There is substantial evidence suggesting that the voltage-dependent anion channel-1 (VDAC1) is a critical player in apoptosis by regulating the release of apoptogenic proteins from mitochondria in mammalian cells and interacting with pro- and anti-apoptotic proteins.

Here, we demonstrate that induction of apoptosis by exposing the cells to various treatments and stimuli results in VDAC oligomerization. Staurosporine, cisplatin, curcumin, As_2O_3 , etoposide, H_2O_2 , UV irradiation and TNF- α , while activating mitochondria-mediated apoptosis via distinct mechanisms, all induce VDAC oligomerization (dimers to multimers). Moreover, a direct relationship between VDAC oligomerization and apoptosis, as reflected in the linear correlation between the extent of apoptosis and the level of VDAC oligomerization, was obtained. Apoptosis induction dramatically en-

hances VDAC1 oligomerization regardless of the cell type used, demonstrating that this phenomena is not cell-type specific. In addition, cell death induced by VDAC1 over-expression also results in highly enhanced VDAC1 oligomerization. These findings support our original proposal that oligomeric VDAC1 forms a structure which mediates the release of cytochrome *c*. We propose that VDAC1 oligomerization is a dynamic process in which apoptosis induction shifts the VDAC1 equilibrium towards oligomerization, forming a large pore allowing the release of apoptogenic proteins, such as cytochrome *c*.

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A Stochastic Pi-calculus Model for the Intrinsic Apoptotic Pathway

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An abstract model for the intrinsic apoptotic pathway is presented. It is encoded in the stochastic pi-calculus formalism and has been tested using the SPiM simulator. The model is consistent with the current knowledge about this phenomenon. The use of this formalism allows the construction of abstract models that can be tested through virtual experiments, thus providing the ability to save resources from real experiment-based tests. Furthermore, the formalism has a proved equivalent graphical representation for describing biomolecular processes, allowing those unfamiliar with the computer science formalisms to be able to use it.

The advances in the biological science and the search of the explanations for the behavior of biological processes such as Ageing and Programmed Cell Death (PCD), make us wonder about the possibilities of finding descriptions for these processes that allow us to understand them, in order to be able to reproduce, and even control them. The need of understanding biological processes has encouraged the search for new ways to describe them, since the most common techniques (differential equations) are not suitable enough for this purpose. As result, plenty of new techniques have been developed in many areas of science, some of which are contributions from the computer science theories of processes and concurrency, the process algebras.

The main features of this calculus are the ability to describe: i) interactions and communication between processes through the concept of name-passing; ii) structure dynamic changes in processes through mobility; and iii) stochastic behaviour by the use of a stochastic semantics. Among the advantages of this formal language for describing biological processes is the ability to test the model without actually building it physically, thus saving resources from its construction until the model has been theoretically proved to be satisfactory.

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Monte Carlo Simulation Shows Noisy Signaling In Apoptosis Increases Risk Of Diseases

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We develop a generalized reaction class based Monte Carlo model to study signaling behavior in apoptotic cell death. We show that apoptotic signaling is noisy under weak stimuli and certain other conditions, which can explain slow apoptotic cell death as observed in recent experiments. Characteristics of such noisy signaling are large cell-to-cell stochastic fluctuations and a bi-modal probability distribution for activated downstream signaling molecule caspase-3. Our study shows how genetic mutations and cell-to-cell stochastic fluctuations in apoptotic signaling can together increase risk of diseases such as cancer. Presence of a specific signaling molecule in the apoptotic pathway and its concentration are often cell type specific. This proposed Monte Carlo model is flexible so that it can be modified to include additional signaling species as well as inhibitors of the apoptotic signaling pathway. Hence, one can readily use our computational model to estimate increased risk of diseases due to faulty apoptotic signaling under various cell-type specific genetic mutations.

Protein Dynamics III

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A Dynamics Criterion to Determine Allostery

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Dynamics coupling, correlated motion and allosteric cooperativity appear to be conserved in the long range communication and conformational transitions of